Neuronal Cell Damage Due to Ionizing Radiation: Fact or Fiction?

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Radiation-Induced Cognitive Deficits

- Occur at doses below threshold for necrosis
- Occur in 25-30% of adults treated with therapeutic ionizing radiation
- Severe problem in children <7 years of age

Initial Questions

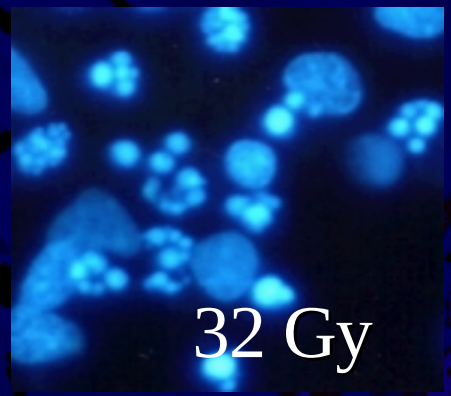
- Does ionizing radiation directly damage neurons?
- What is the mechanism for such an effect?
  - Biophysical Basis
    - Free Radicals
    - Direct Damage
  - Molecular Target(s)
    - Lipid Peroxidation
    - Protein Oxidation
    - DNA Damage
  - Downstream Signals and Effectors

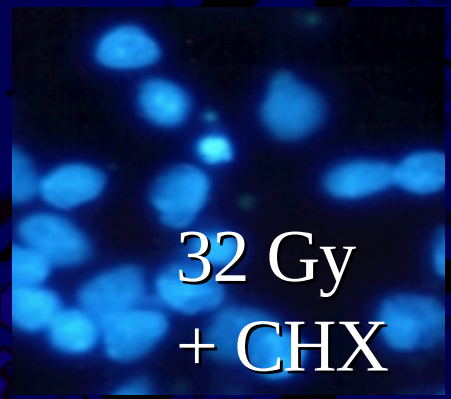
Materials and Methods

- System - Rat Neuron Culture (E16-18)
- Conditions - Neurobasal Media + B27
- Treatment(6 Days after Isolation)
  - "Moderate" Dose Irradiation (<32 Gy)
  - "High" Dose Irradiation (>64 Gy)
- Evaluation
  - Nuclear Morphology - Hoechst (12 h)
  - Membrane Permeability - LDH Release (18 h)
  - Viability - Calcein AM Staining (24-48 h)

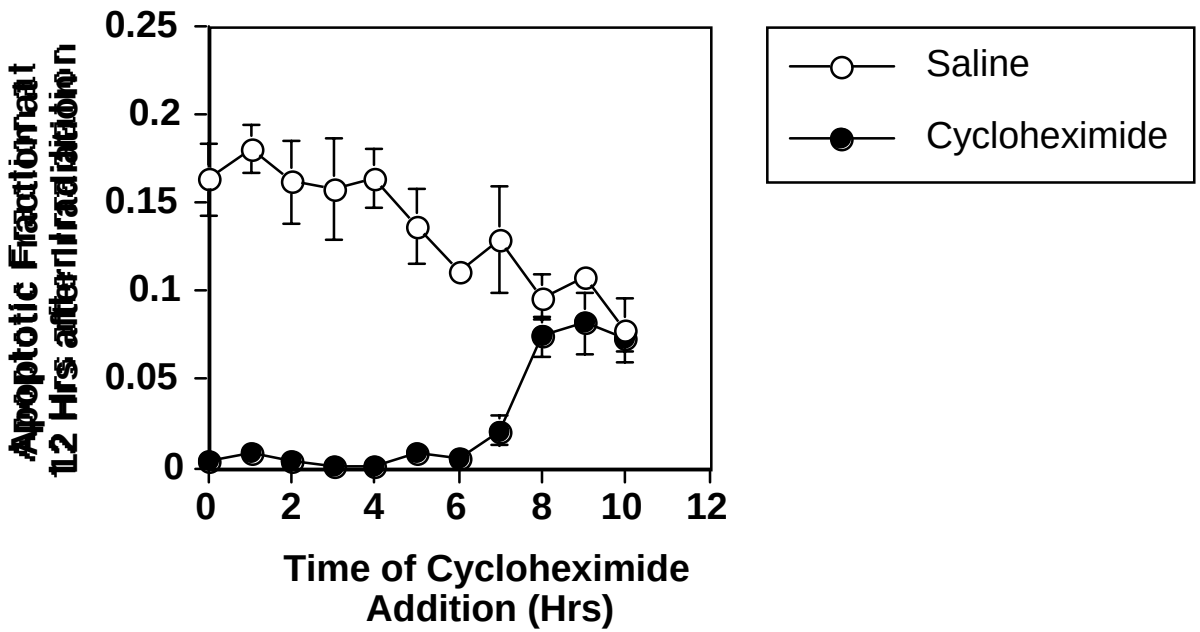
Neurons and Low Dose Irradiation

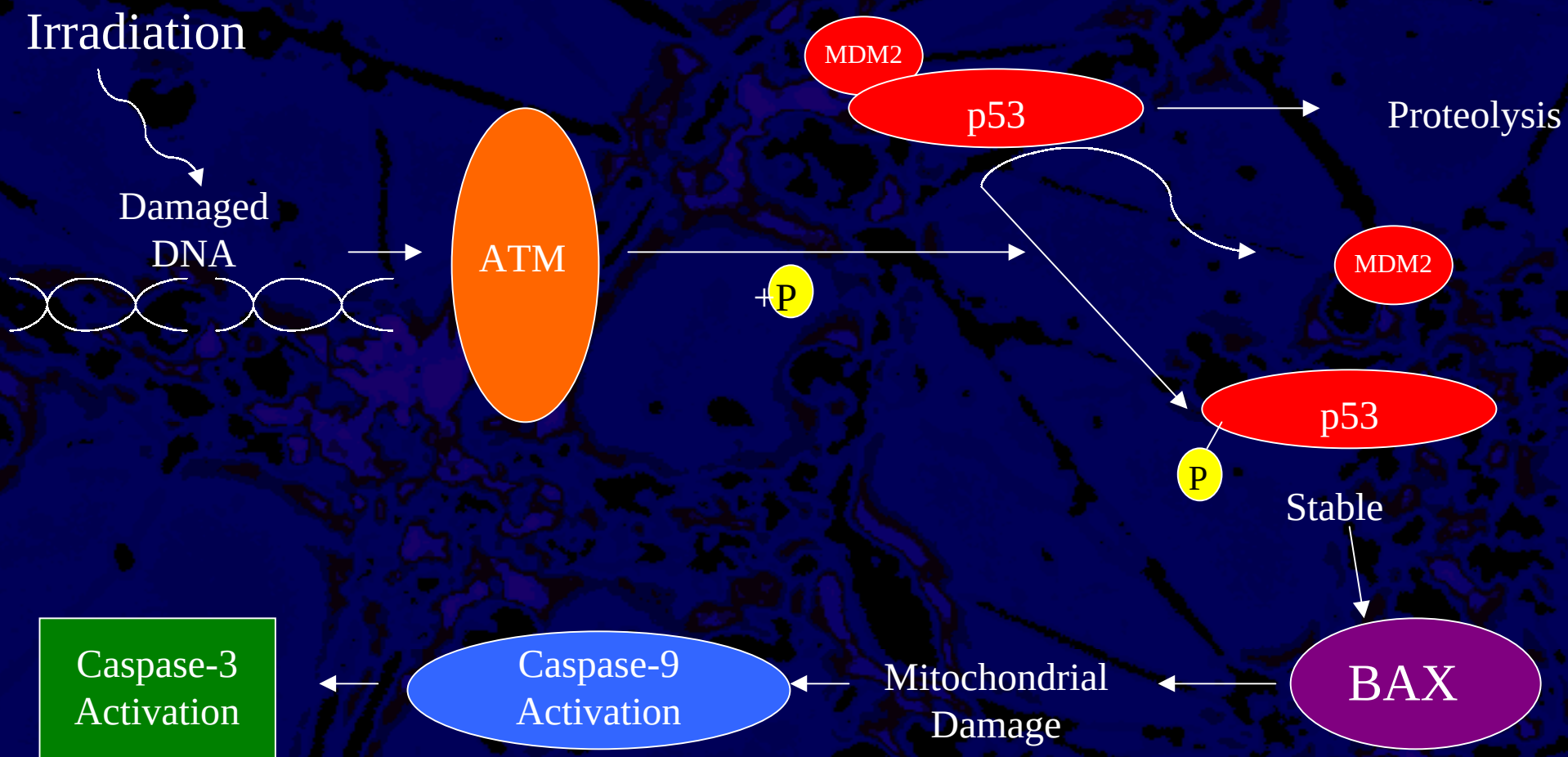

0 Gy


32 Gy

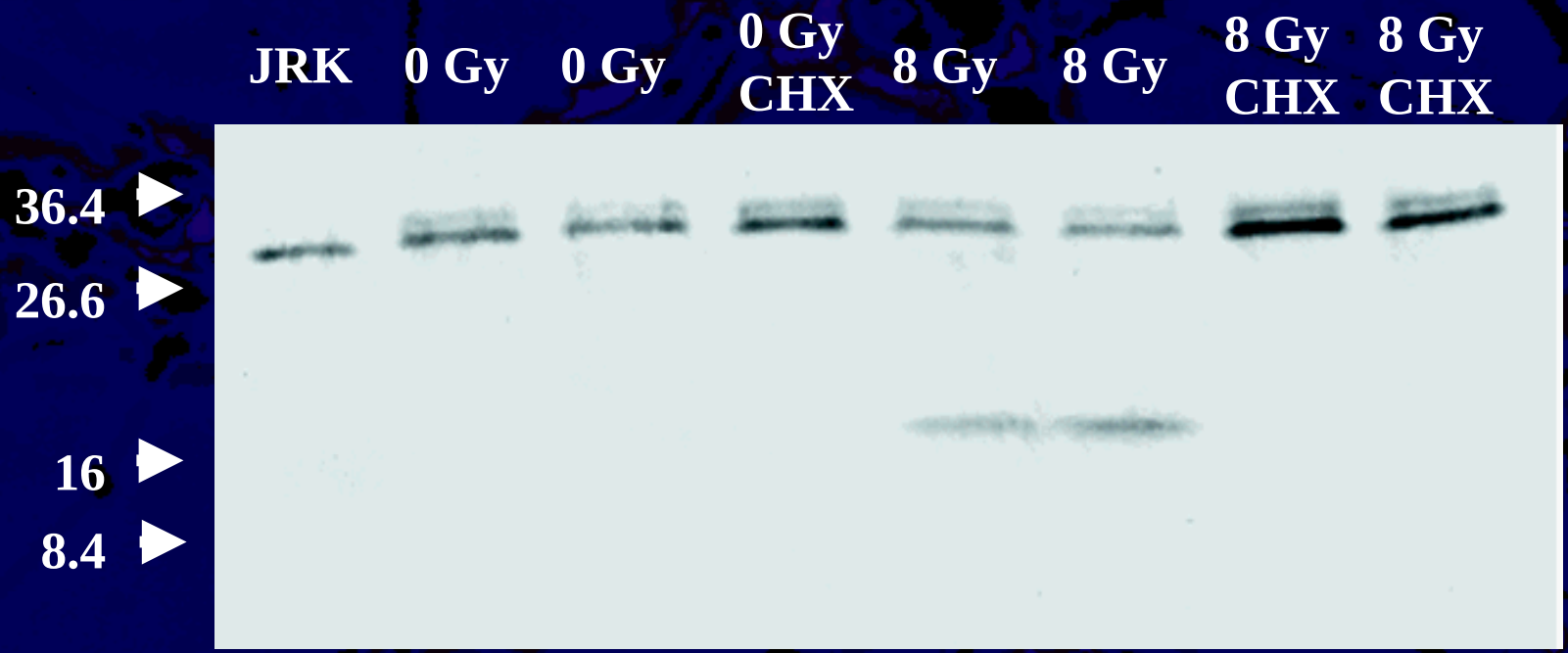

32 Gy
+ CHX


0
32
STD

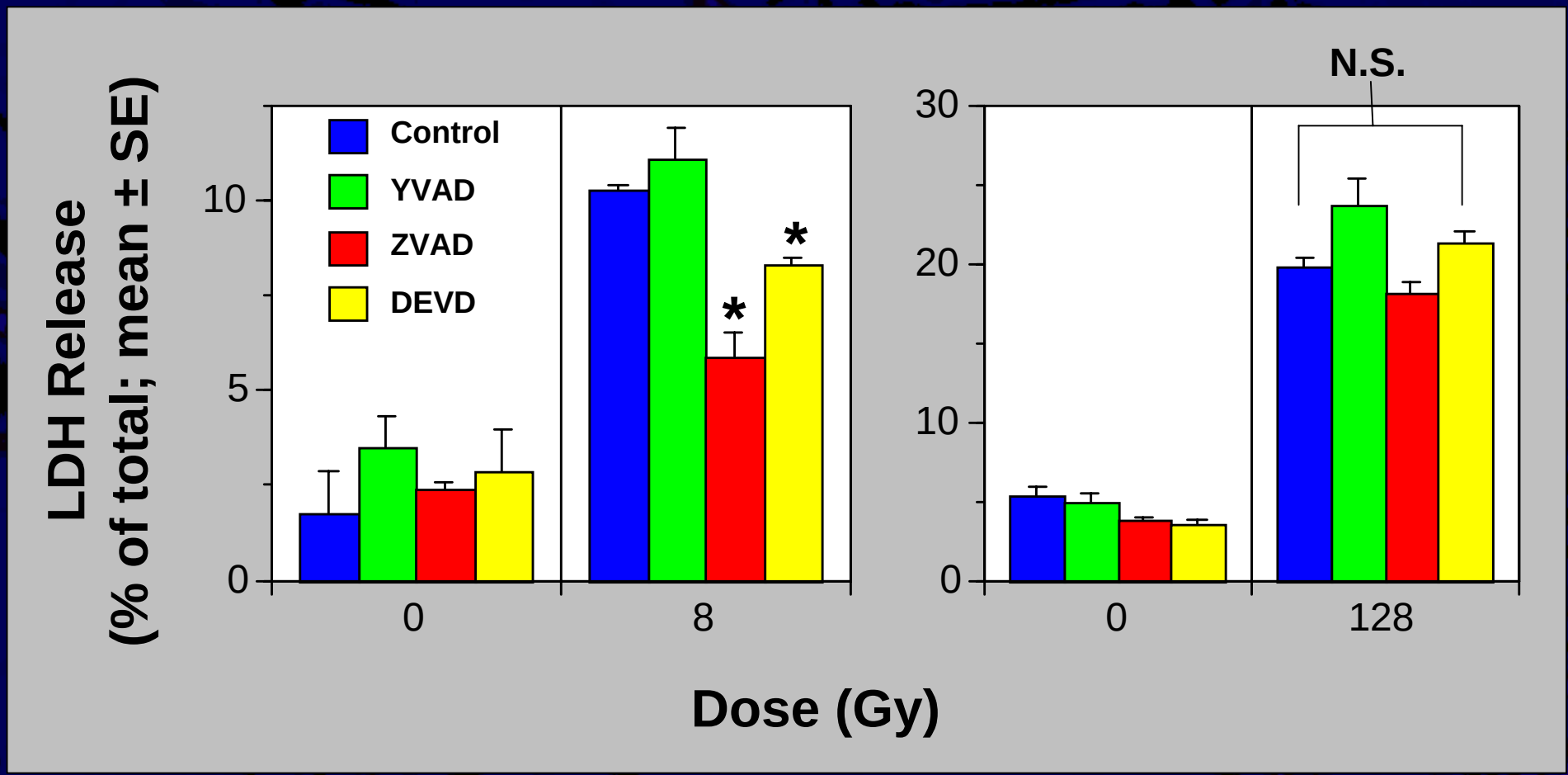

Apoptotic Fraction at
12 Hrs after Irradiation
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
12
Time of Cycloheximide
Addition (Hrs)
Saline
Cycloheximide


Irradiation
Damaged DNA
ATM
+P
MDM2
p53
Proteolysis
MDM2
p53
P
Stable
BAX
Mitochondrial Damage
Caspase-9 Activation
Caspase-3 Activation

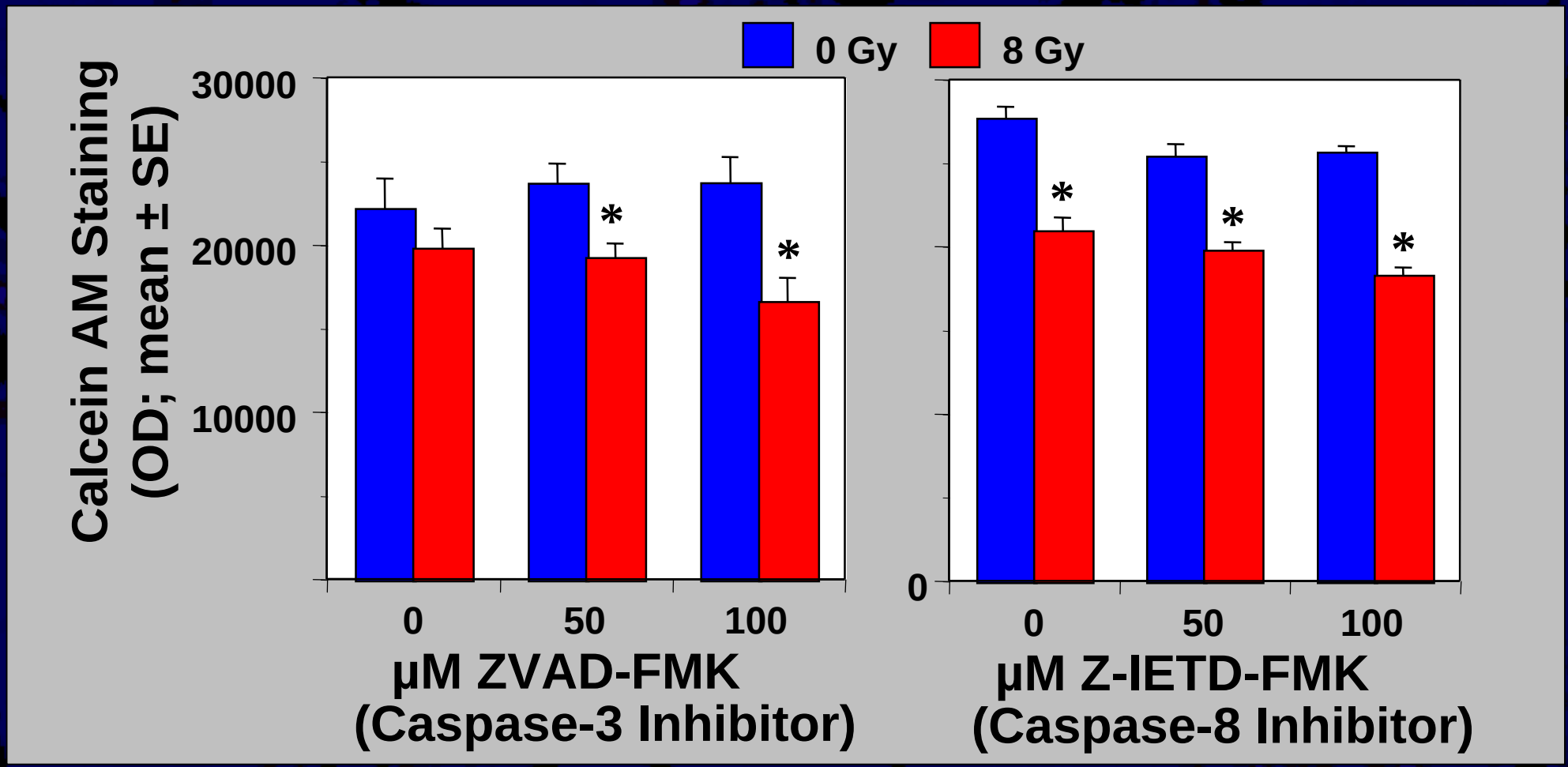
Cycloheximide Blocks Activation of Caspase-3



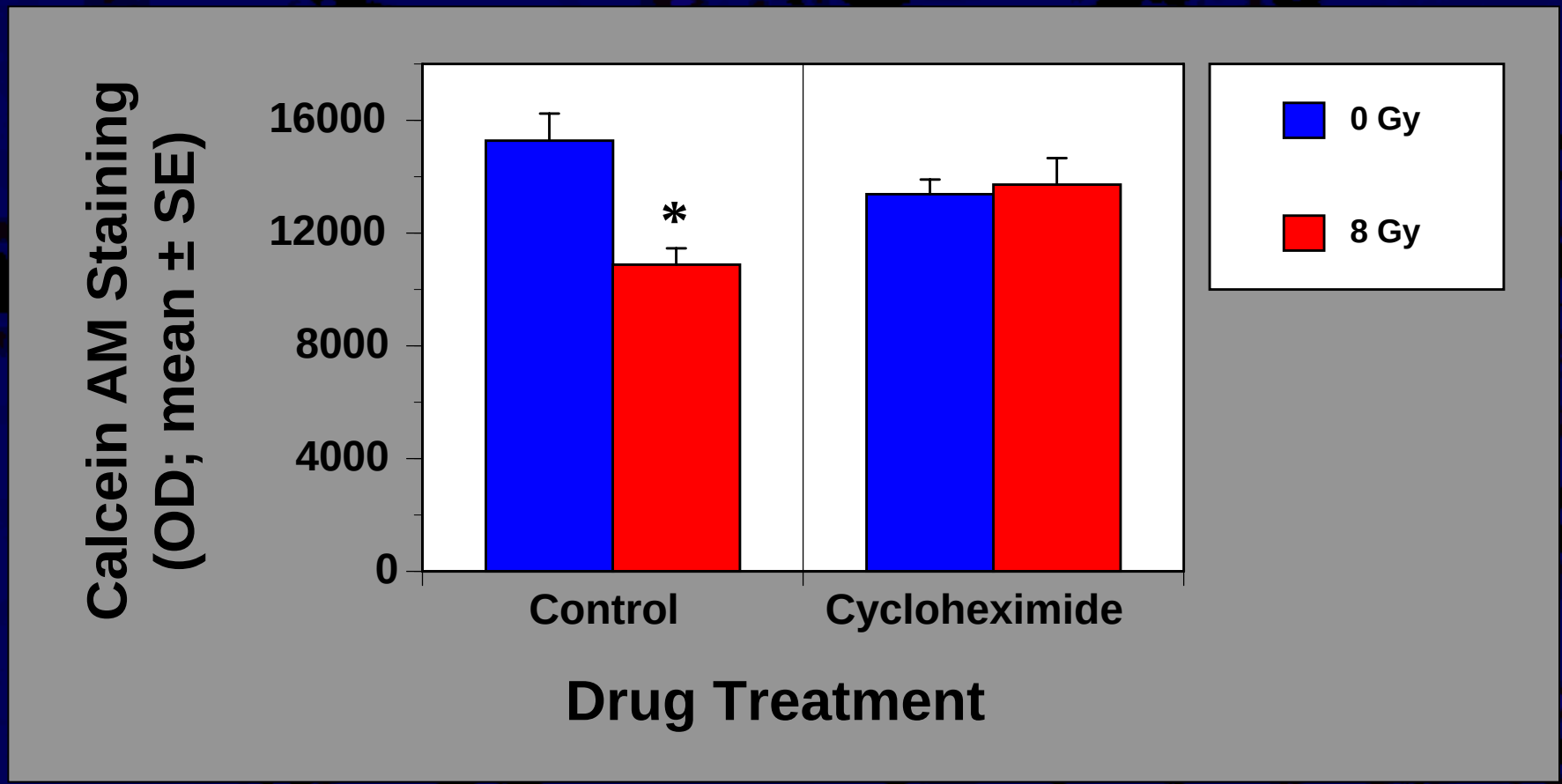
Caspase Inhibitors Block Loss of Membrane Integrity due to Apoptosis



Caspase Inhibitors Do Not Block Loss of Viability After X-Rays



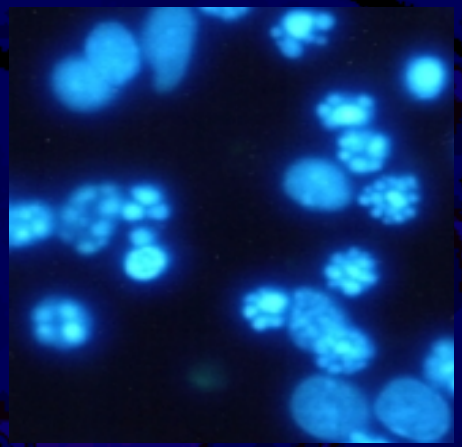
Cycloheximide Does Block Loss of Viability following DNA Damage



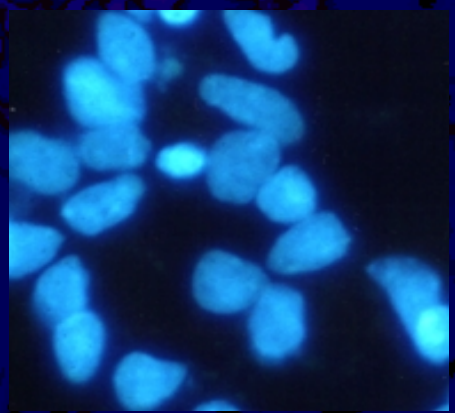
Neurons and High Dose Irradiation

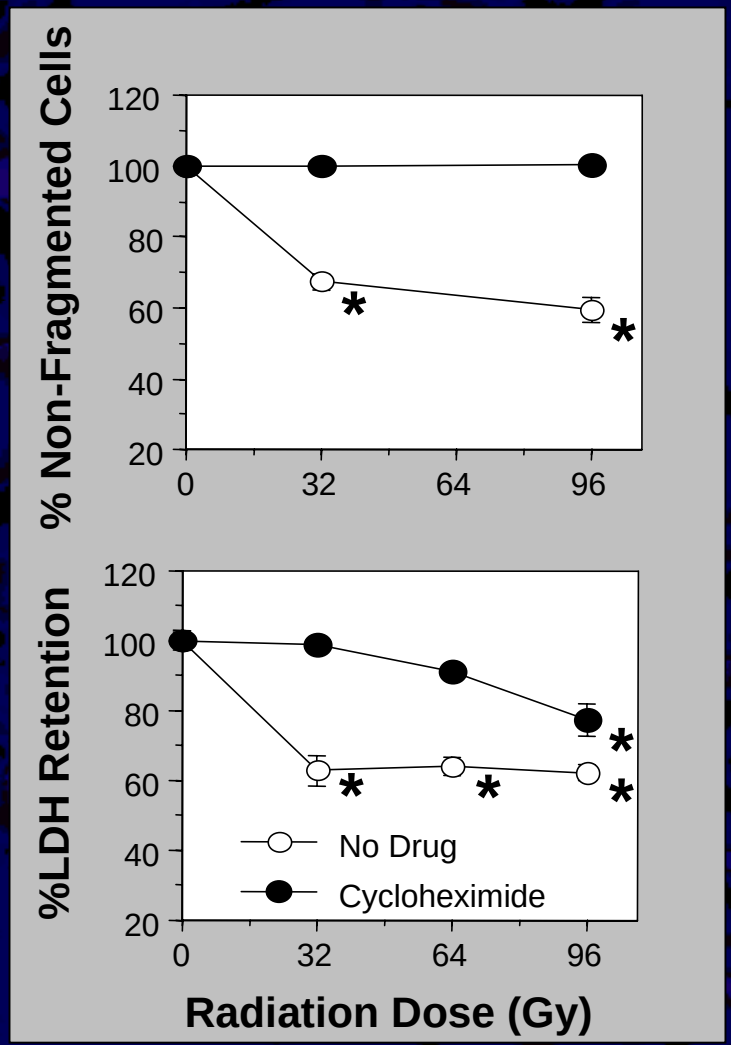
Response to High Doses (>32 Gy)

96 Gy



96 Gy
+ CHX



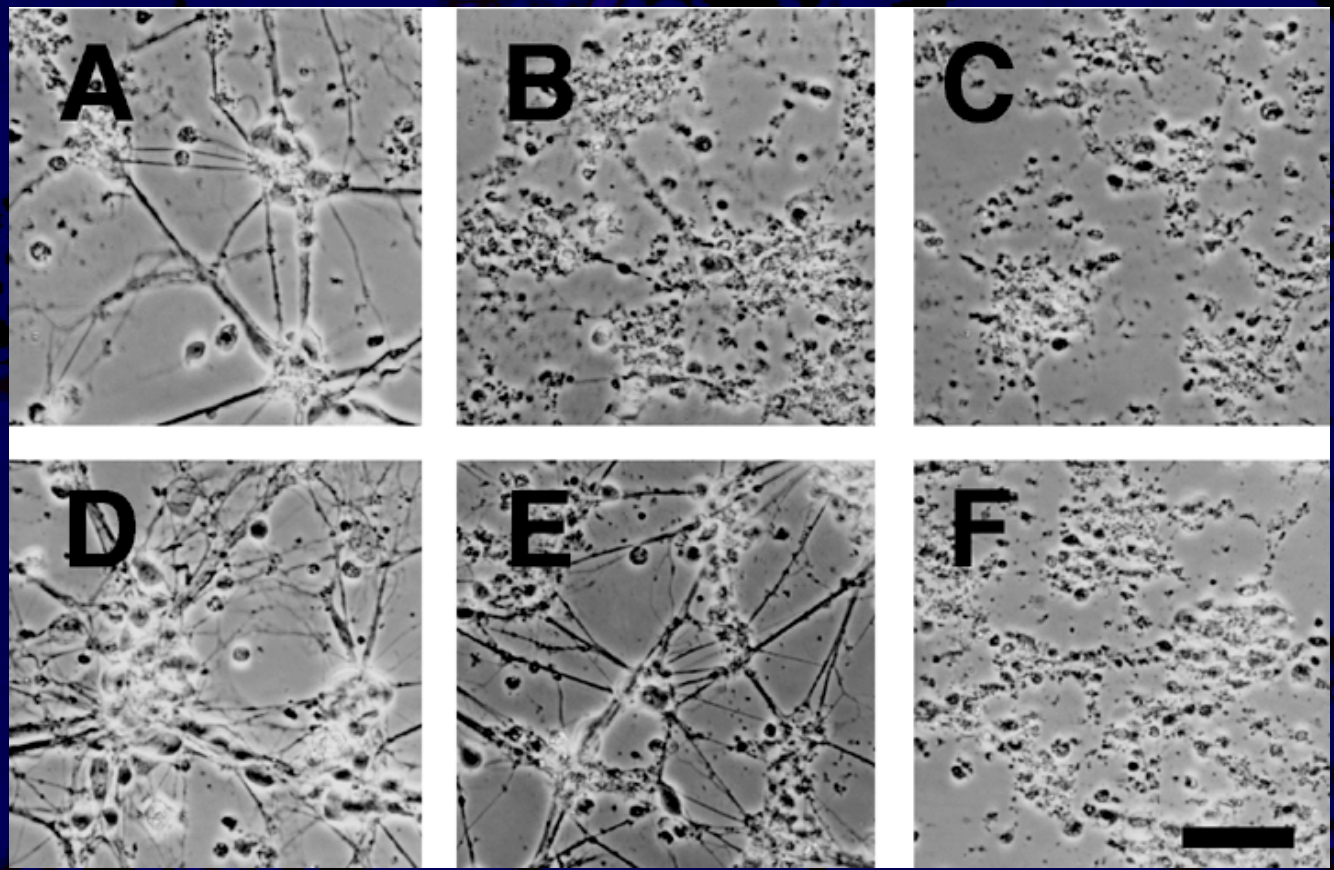


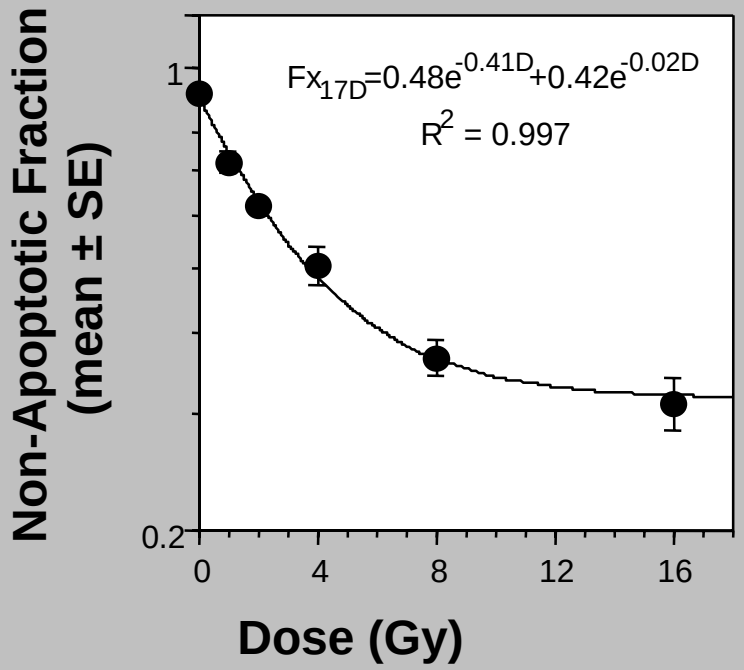
Neuron Morphology 24 h after 0-96 Gy ± Protein Synthesis Inhibitor (CHX)

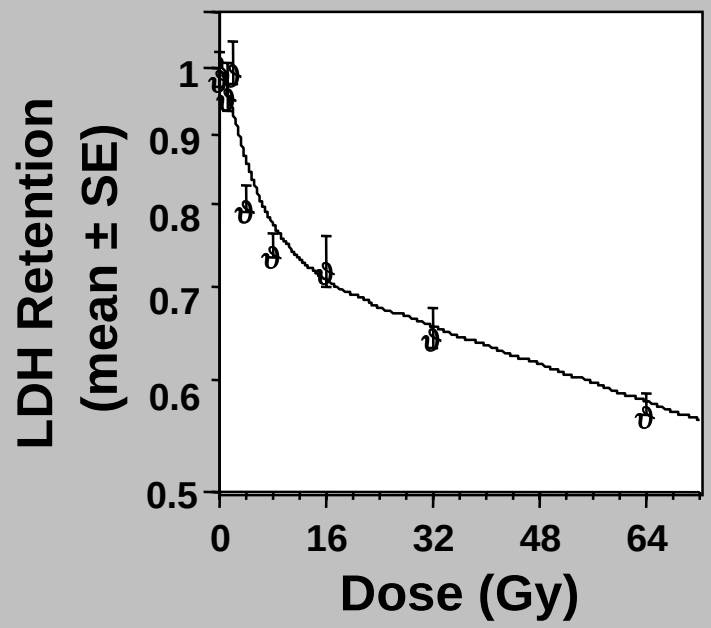
0 32 96

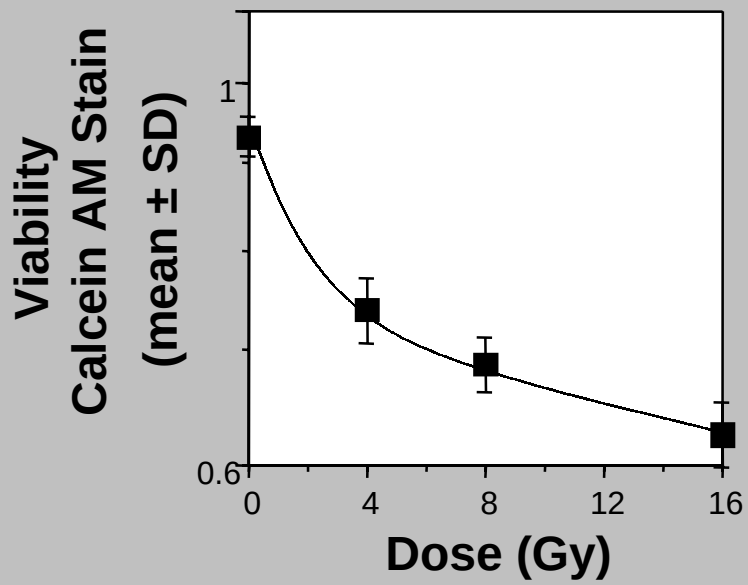
-CHX

+CHX

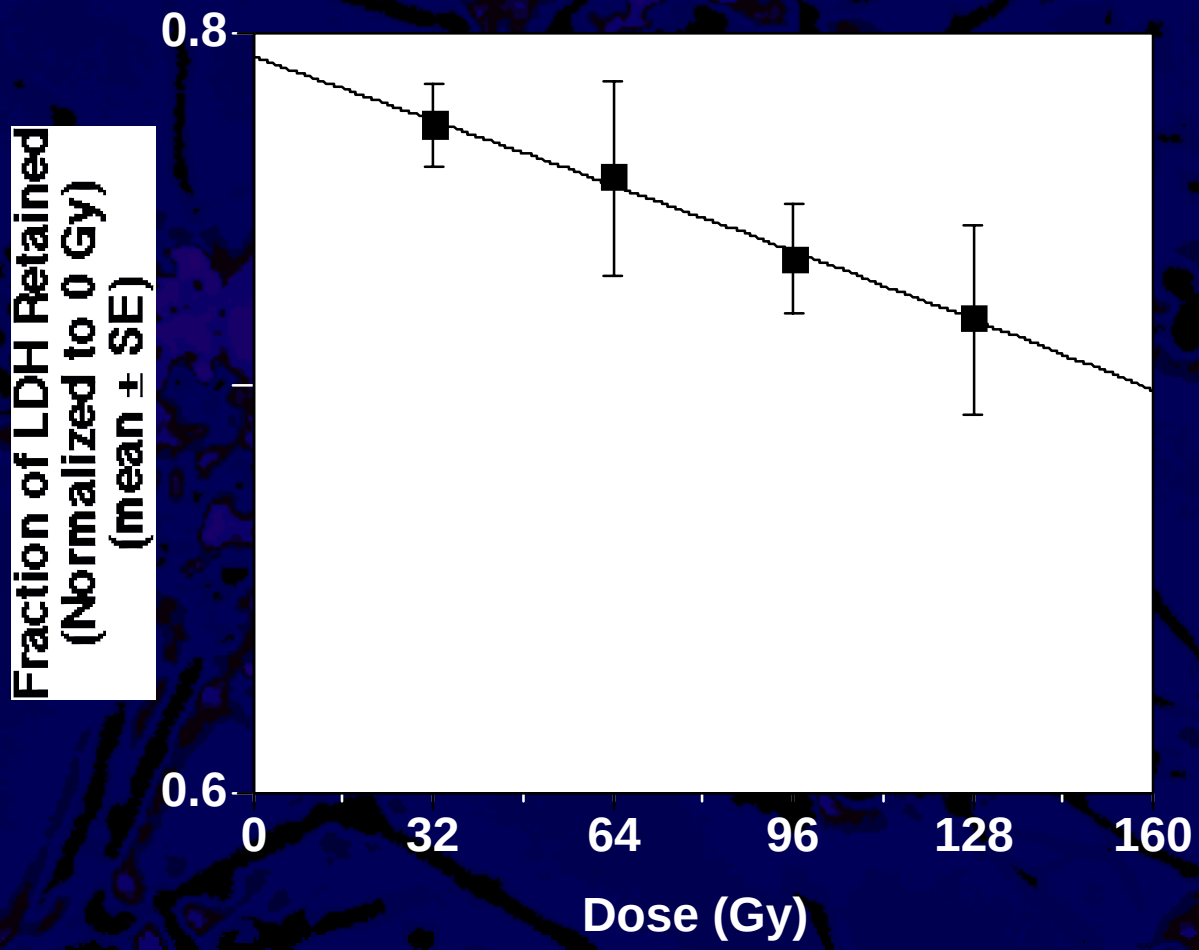



Non-Apoptotic Fraction
(mean ± SE)
$Fx_{17D}=0.48e^{-0.41D}+0.42e^{-0.02D}$
$R^2 = 0.997$
1
0.2
0
4
8
12
16
Dose (Gy)

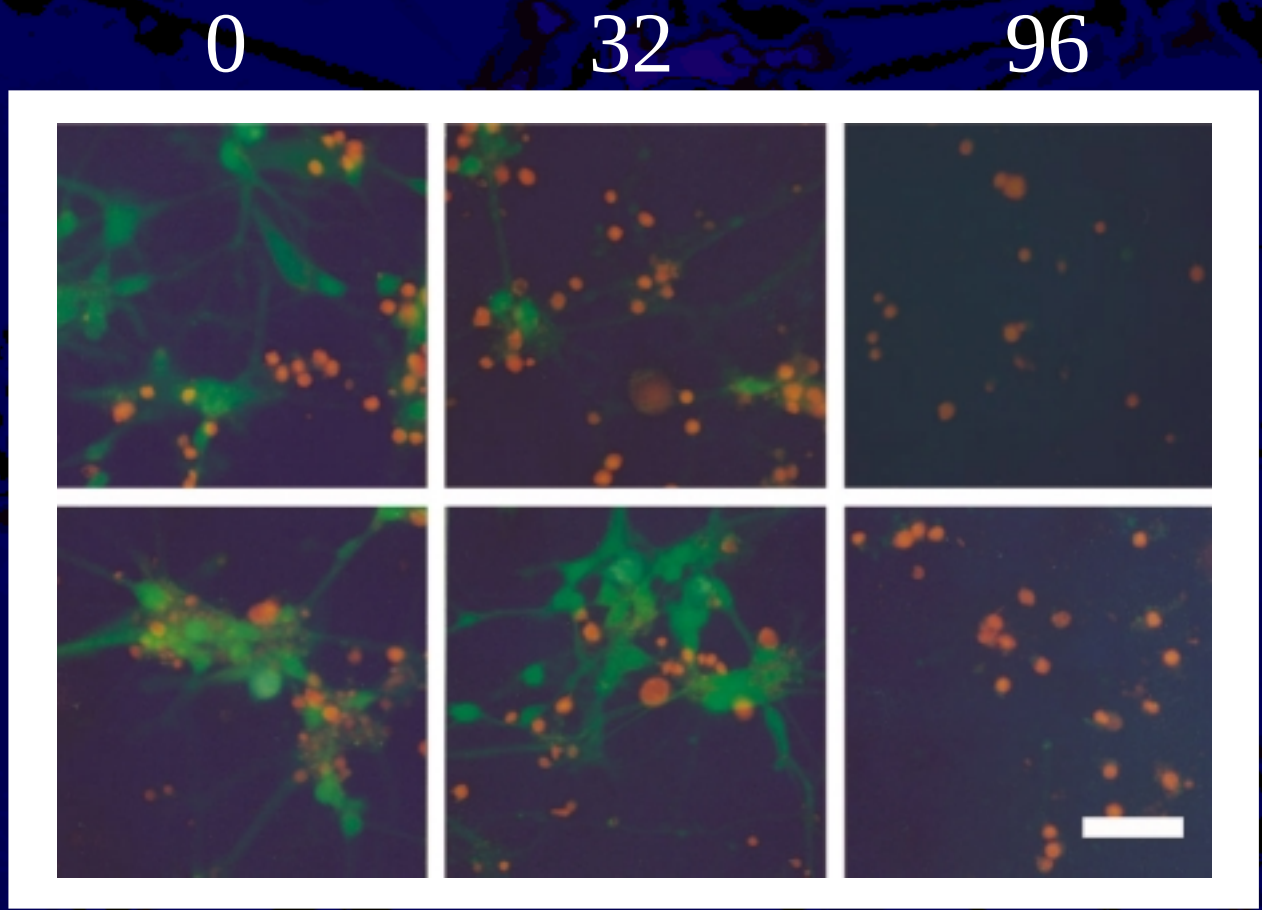

LDH Retention
(mean ± SE)
1
0.9
0.8
0.7
0.6
0.5
0
16
32
48
64
Dose (Gy)


Viability
Calcein AM Stain
(mean ± SD)
1
0.6
0
4
8
12
16
Dose (Gy)

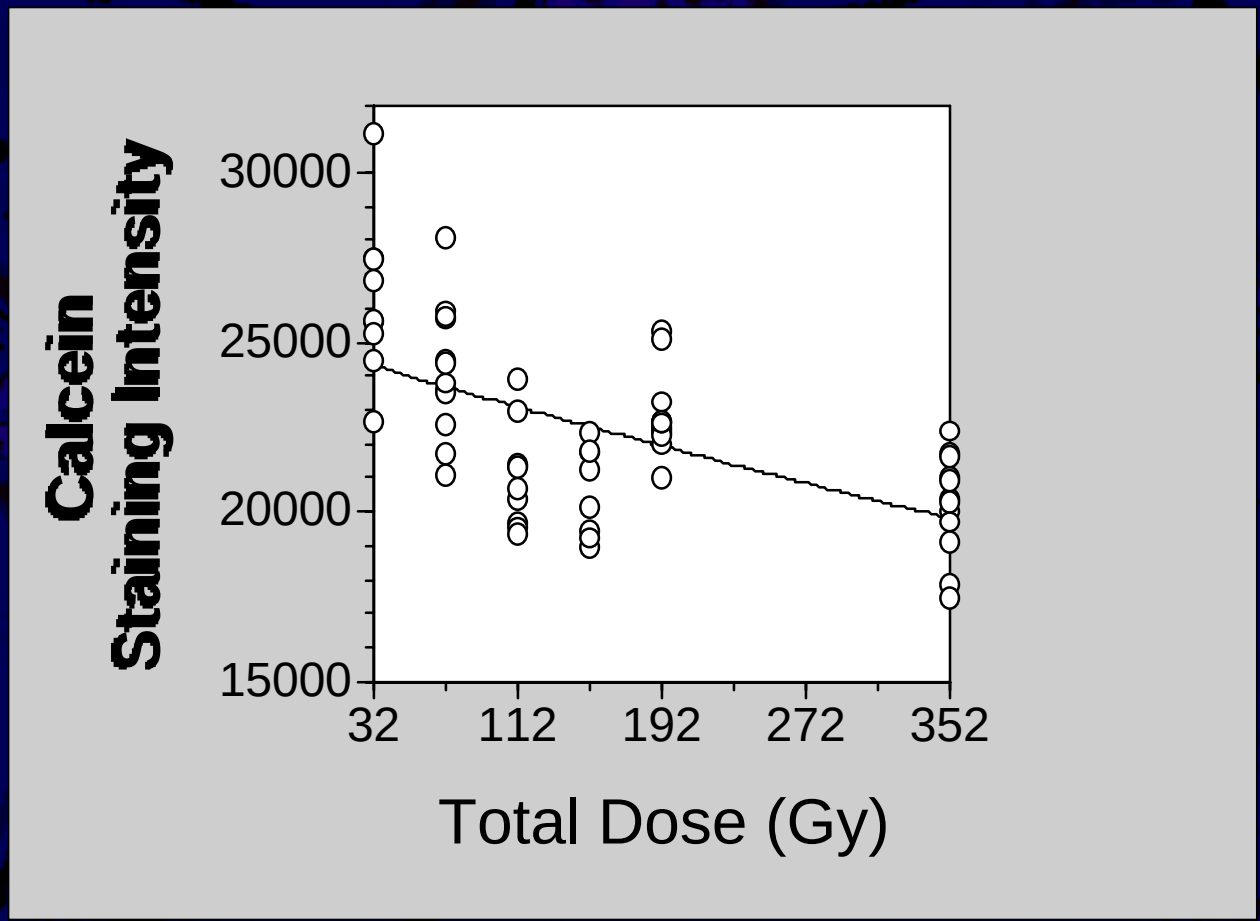
Effect of Single Dose X-Irradiation on LDH Retention



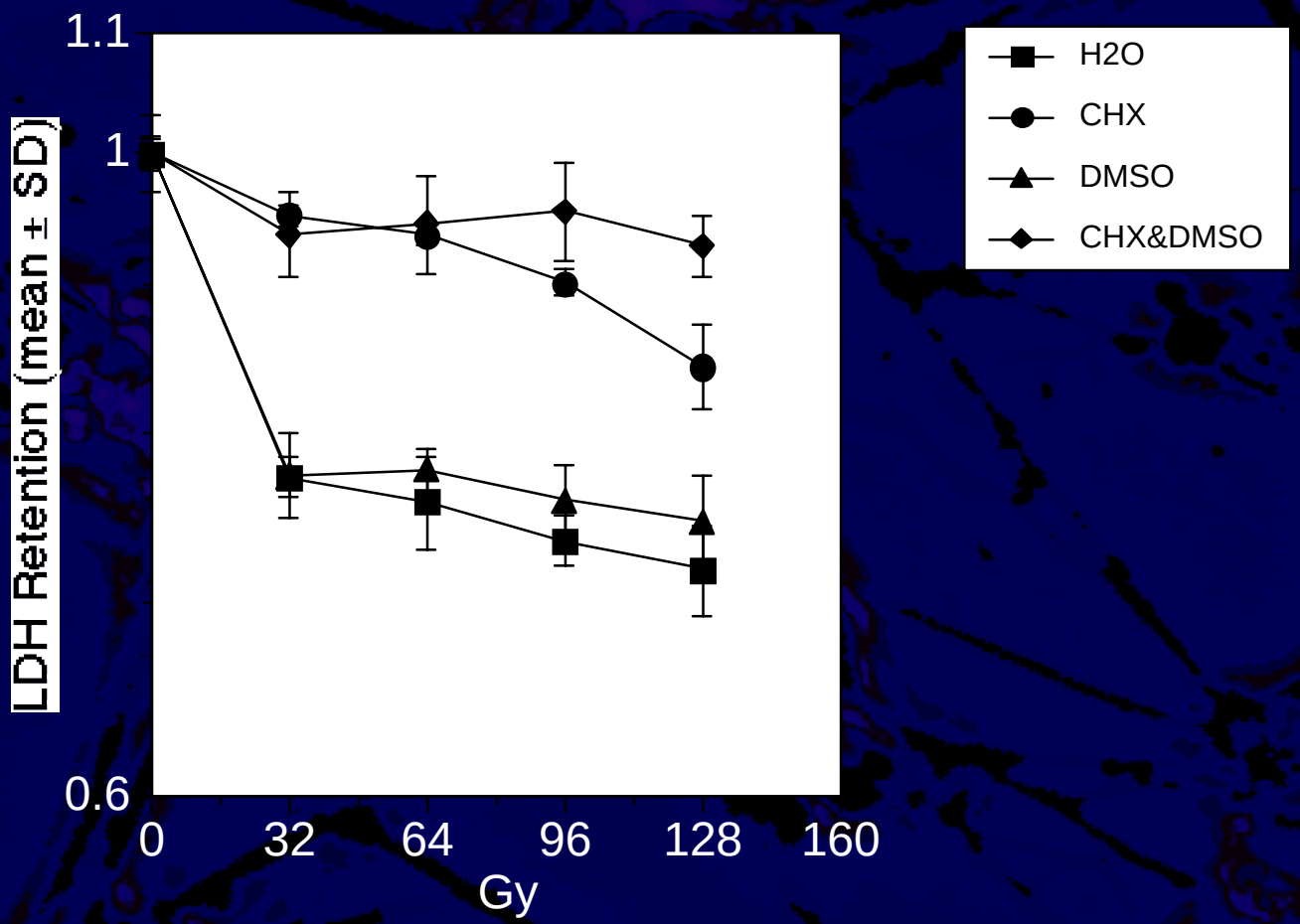
Neuron Viability 24 h after 0-96 Gy ± Protein Synthesis Inhibitor (CHX)



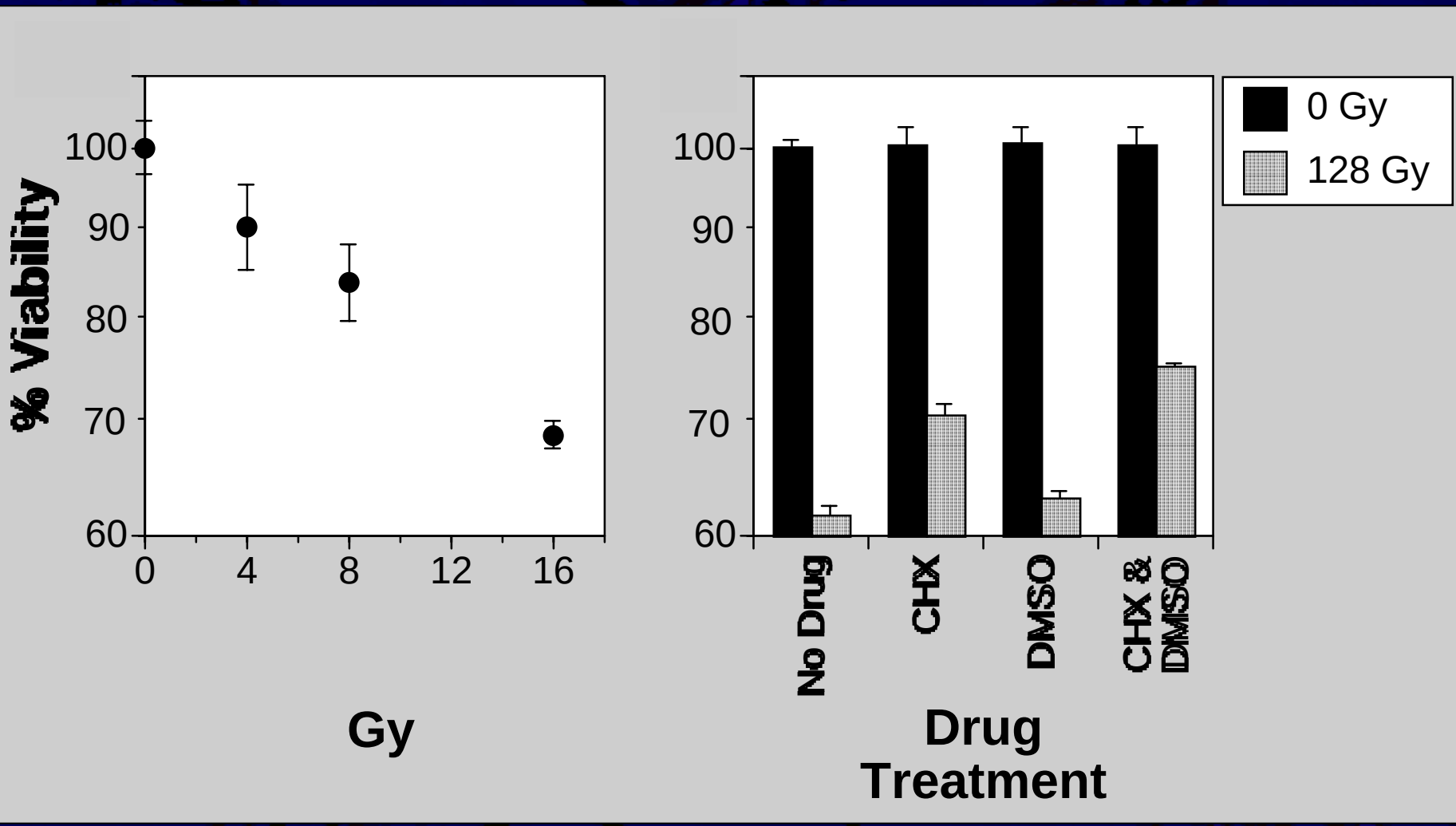
Effect of High Dose Fractionated Irradiation on Neuron Viability



Effect of DMSO on Membrane Integrity after Ionizing Radiation



DMSO and Viability after High Dose X-Irradiation



Effects of Antioxidants on Neuronal Damage After Ionizing Radiation

Antioxidant	*Apoptosis Effect (4 Gy)*	*Necrosis Effect (96 Gy+CHX)*
DMSO	None	Moderate
Mannitol	Slight Reduction	None
Superoxide Dismutase	None	ND
Catalase	None	ND
Trolox	None	None
Acetylcysteine	ND	None

Summary

- Low Dose X-rays -> Immature Neuronal Apoptosis
- Caspases & Apoptosis - Protein Synthesis Dependent
- High Dose X-rays -> Non-apoptotic Neuronal Death
- DNA Damage Most Likely Cause